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Behavioural Evidence for Polychromatic Ultraviolet Sensitivity in Mantis Shrimp

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POPULAR SUMMARY

Mantis shrimp have spectacularly sophisticated eyes, with a number of unique elaborations that stretch their visual capabilities far beyond our own, including deep into the ultraviolet (UV) range. Bok *et al*., 2018 uses trained and innate behavioural response experiments to show that mantis shrimp are able to detect and discriminate various UV stimuli. Most notably, they respond differentially to stimuli in the near-UVB (< 315 nm in wavelength) versus longer-wavelength UVA stimuli. These UVB cues lie outside the discriminable range of most other animals and could afford the mantis shrimp yet another covert visual signalling domain.

KEYWODS: Ultraviolet vision, Visual ecology, Mantis shrimp, Colour vision

ABSTRACT

Stomatopod crustaceans are renowned for their elaborate visual systems. Their eyes contain a plethora of photoreceptors specialized for chromatic and polarization detection, including several that are sensitive to varying wavelength ranges and angles of polarization within the ultraviolet (UV) range (< 400 nm). Behavioural experiments have previously suggested that UV photoreception plays a role in stomatopod communication, but these experiments have only manipulated the entire UV range. Here, using a behavioural approach, we examine UV vision in the stomatopod *Haptosquilla trispinosa*. Using binary trained choice assays as well as innate burrow choice experiments, we assessed the ability of *H. trispinosa* to detect and respond to narrow-band LED stimuli peaking near 314 nm (UVB) versus 379 nm (UVA) in wavelength. We find that *H. trispinosa* can discriminate these stimuli, and appears to display an aversive reaction to UVB light, suggesting segregated behavioural responses to stimuli within the UV range. Furthermore, we find that

H. trispinosa can discriminate stimuli peaking near 379 nm versus 351 nm in wavelength, suggesting that their wavelength discrimination in the UV is comparable to their performance in the human-visible range.

BACKGROUND

Stomatopods, or mantis shrimp, are well known for their aggressive predatory behaviour, sophisticated social interactions, and colourful markings (**Figure 1A**) [1]. However, they have drawn the most extensive scientific interest for their unusual and complex visual systems. Their eyes are modified from typical malacostracan dichromatic, apposition compound eyes by a midband of specialized ommatidia that horizontally bisects each eye (**Figure 1B**). Within this midband region, the photoreceptors are structurally and physiologically adapted for the detection and discrimination of eight colour bands within the human-visible range (400-700 nm), as well as linearly- and circularly-polarized light [2-5]. Furthermore, the midband contains up to five types of ultraviolet (UV) photoreceptors, maximally sensitive to various wavelength ranges of light below 400 nm [6-8], including a pair of UV-linear-polarization-sensitive photoreceptors [9]. These UV photoreceptors are uniquely tuned to narrow wavelength ranges of UV light by filtering pigments in the optical elements of the ommatidia derived from biological sunscreen compounds [10-12], suggesting the potential for chromatic discrimination in the UV range.

Despite a robust understanding of stomatopod retinal physiology, little is known about how visual information is processed and employed to initiate or mediate behavioural responses. Behavioural trained choice foraging experiments have been performed at human-visible wavelengths in order to demonstrate sensitivity to colour [13, 14], linear polarization [15, 16], and circular polarization [5]. Furthermore, results of burrow-choice experiments have suggested that sensitivity to circularly polarized light may play a role in in intraspecific communication [17]. Behavioural assays that test the UV range (< 400 nm) are thus far limited to antagonistic encounter experiments that suggest stomatopods assess UV cues in territorial contests [18]. However, these experiments manipulated the entire UV range and did not examine the contribution of individual UV receptor spectral types to stomatopod behavioural responses. It is not known whether the multiple UV photoreceptor classes found in the stomatopod eye are used to make spectral discriminations.

The stomatopod *Haptosquilla trispinosa* has at least three spectral classes of UV-sensitive photoreceptors located in the eighth retinular cells (R8s) of the midband (**Figure 1C-D**) [14]. Two of these photoreceptor classes have segregated spectral sensitivity curves, with one photoreceptor class responding to

light primarily in the UVA range (315-400 nm), and a second absorbing light strongly in the UVB range (≤ 315 nm). Based upon these electrophysiological spectral sensitivity measurements, we hypothesized that *H. trispinosa* would be capable of detecting and discriminating UVA versus UVB stimuli. Here we present the results of trained predatory choice and innate burrow preference behavioural experiments in *H. trispinosa.* We show that these stomatopods are able to discriminate and behaviourally respond to UVA and near-UVB stimuli. Furthermore, our results suggest spectrally distinct roles for UVA versus UVB cues in stomatopod behaviour, possibly related to intraspecific communication.

MATERIALS AND METHODS

Animals

Haptosquilla trispinosa individuals were collected at the Lizard Island Research Station (Queensland, Australia, 14°40'43.9"S, 145°26'47.9"E) at 1 meter depth in May and June of 2012 (for trained choice tests) and in June 2014 (for the innate burrow preference tests). The individuals used in the trained choice experiments ranged in length from 23-36 mm, with a mean of 28.9 mm. The individuals used in the innate burrow choice experiments ranged in length from 21-37 mm, with a mean of 28.7 mm. They were kept for 1-5 days in individual cups with daily water changes and regular feeding with small pieces of snail or crustacean meat until they were moved to the training or experimental setups.

Trained Choice Tests

The trained choice test followed a similar approach to that of Thoen et al. [14]. The animals were housed in individual aquaria with artificial burrows constructed from plastic vials positioned in a sand bed and with constant seawater flow-through. The training and experimental apparatus was custom constructed by John Cataldi at the University of Maryland, Baltimore County MME Technical Service Center Machine Shop. It consisted of pair of submersible targets with a 3 mm hole in the centre connected to an above-water light emitting diode (LED) mount and controller by a pair of 10-cm-long, 3 mm diameter optical guides in blackened brass tubes (**Figure 1E, S1**). The stimuli were generated using one of three LEDs with maximum emission (λ_{max}) at 314.3 nm (UVB), 378.3 nm (UVA) and 351.1 nm (UVA-351) (**Figure 1E, inset**). Note that UVA refers to the 378.3 nm LED stimulus unless otherwise indicated. Wavelengths of light beyond 400 nm were blocked by a UV bandpass filter in the target head. Refer to **Figure S1** for diagrammatic representations

and technical details of the testing apparatus and the stimuli. The brightness of the LEDs were modulated by a custom controller (**Figure S1E, S2A-B)**.

The trained choice test exploited an innate predatory behaviour where *H. trispinosa* will lunge from its burrow to attack a target. In the choice test trials, the animals were presented with a pair of targets and trained to associate a food reward (shrimp or mollusc meat) with a UVA or UVB stimulus. Possible confounds in brightness were controlled for by modulating the relative intensities of the two LED stimuli in the trials. The intensity of each LED was randomized between five intensity settings in the UVA vs. UVB experiments (Figure S2A) and between four settings in the UVA vs. UVA-351 experiment (Figure S2B).

Prior to the trials, individuals were first made accustomed to feeding from a single practice target, presented alone and without any emitted UV stimuli. The underside of these training targets had a small groove, not visible from the front, that a small piece of snail meat could be affixed to. The choice trial targets used in the subsequent experimental rounds did not have this groove and never came in contact with food. Individuals that learned to feed from the single training target were then split into cohorts of initially 12 randomized individuals, roughly balanced for even distributions of gender and size, to begin choice training. The animals in each cohort were trained to associate a particular stimulus with a reward in a randomized binary choice context against a second alternate stimulus. For training, a piece of food was affixed to the underside of the correct training target. The two targets were positioned in the water, at a distance from the burrow necessitating the animal to lunge fully from the burrow in order to collect the food. Once the animals were feeding from the correct stimulus consistently, choice test trials were initiated.

Trials were carried out by first blocking the burrow entrance with an opaque plastic sheet so that enthusiastic individuals were prevented from leaving the burrow until the targets were positioned properly. Once the targets were in place, seawater that had contained thawing reward food (shrimp or snail muscle) was poured broadly over the front of the sheet to alert and stimulate the animals with an odorant cue. The plastic sheet was then lifted, and the animals were given two minutes to make a choice. Choices were scored when an animal fully exited the burrow and touched one of the targets. Correct choices were rewarded by giving the animal a piece of food on a feeding stick. Incorrect choices terminated the trial for that individual and the targets were removed. If no choice was made, it was noted whether the animal extended its head from the burrow to assess the target but never attacked, or did not emerge at all (**Figure S1B)**. The experimenter observed the trials on a camera viewscreen from behind masking material mounted on the LED controller.

Ambiguous responses were not rewarded, but were reviewed and scored from the recorded video. If the animal did not clearly hit a target, or if it lunged between the targets, it was scored as a failure to participate.

Trials were carried out three to five times per day until each cohort reached 30 trials. Intermittent binary training rounds were performed (as described above) once or twice a day, usually before the first trial round of the day, in order to motivate and reinforce the behaviour. The cohort that was trained to choose UVA from UVB was used for control experiments where they were presented with two identical UVA stimuli. This same cohort was then used in an additional 30 trial experiment that asked the individuals to continue choosing the UVA stimulus, but now against the UVA-351 stimulus. The initial experiments involving cohorts 1, 2, and 3 (UVA vs. UVB, UVB vs. UVA, and UVB vs. dark) were carried out simultaneously. A second set of experiments involving cohorts 4 and 1 (dark vs. UVB, and UVA vs. UVA-351) were performed simultaneously following the initial three. During the experiments, trials were performed throughout the day and we alternated from one cohort to another after each trial round.

Upon the completion of the trials, correct, incorrect, and non-participatory outcomes were collated for each individual (Table S1). An individual did not participate (DNP) if it assessed the targets but made no choice. All individuals who never made a choice in a trial were removed from the dataset. In some cohorts, this created the gender ratio imbalance reported in Table S1. The percent correct choices and percent participation values for each individual were then averaged within each experimental cohort in order to preform statistical analysis without pseudoreplication (**Table 1**, see additional details about statistical analysis below).

Innate Burrow Preference Tests

The second set of experiments exploited innate cover-seeking behaviour in *H. trispinosa*. Naive animals, assorted into four groups with roughly equivalent gender and body length distributions, were introduced into the centre of a circular arena facing a pair of artificial burrows that emitted either UVA (λ_{max} = 379.1 nm), UVB (λ_{max} = 317.4 nm), or no light stimuli (**Figure 1G**). Note that these stimuli differ slightly in spectral properties from those in the trained choice tests despite being generated by the same LEDs because of different optical components in their respective setups (**Figure S1, S2**). In the experiment that tested UVA versus UVB preference, the LEDs were modulated in order to produce stimuli of equivalent sum radiance (**Figure S2C**). The stimuli were again generated by a pair of LEDs which illuminated a diffuser at the back of

the burrow, and passed through a 1 cm hole covered by a UV bandpass filter into the burrow. The stimuli were randomized between the two burrows for each trial. The arena had a sand bottom and continuous water flow-through entering from behind the animals and exiting through blackened air line tubes emerging through the bottoms of the two artificial burrows. Refer to **Figure S1F-H and S2C** for diagrammatic representations and technical details of the testing apparatus and the stimuli.

For each trial, an animal was loaded into a stoppered clear glass flask and introduced into the centre of the arena with the flask's opening facing the two burrow options. The stopper was removed, and the animal was given up to two minutes to make a choice (it typically required much less time). Trials were observed by the experimenter, positioned outside of the view of the arena, via a camera viewscreen. Choices were counted when an individual moved directly from the flask into one of the burrows. In the event that the animal never left the flask, never entered a burrow, or moved to an adjacent side of the arena before entering one of the burrows, the trial was scored as a failure to participate. The sand at the bottom of the arena was stirred between trials to obscure chemical cues.

Statistical Analysis

All statistical analyses were conducted in R (version 3.3.2 [19]). In the trained choice tests, correct, incorrect, and non-participatory outcomes were recorded for each individual trial and were analysed using generalized linear mixed model (GLMM) (lme4 package [20]). To assess the influence of the wavelength and stimulus brightness on the choice of the individual, a single model was conducted on the binary response variable using the fixed factors of wavelength, relative brightness level, and the interaction between these two factors. We included the individual animal identity as a random term to control of the repeated measures per animal. Single term deletions were used to reduce the model to its minimum form.

As brightness changes were found not to influence the choice of the animals, further analyses for assessing the effect of the wavelength on choices were conducted using a one-sample Wilcoxon test. In each experiment, the repeated results from the multiple trails per individual were averaged to provide a mean proportion for a measure of successes for each individual (**Table S1**). These data were then compared for each experiment against an expected mean of 0.5 (50% correct choices) based on a null hypothesis of the animals not having the capability to differentiate between the different spectral contents of the stimuli (**Table 1**).

For the burrow preference tests, singe naïve individuals were tested once in each trial and a binomial test was used to analyse if their pooled innate responses differed due to the different burrow illumination stimuli.

RESULTS

Trained Choice Tests

Six experiments were performed testing the ability of *H. trispinosa* to learn to discriminate and choose UVA (λ_{max} = 378.3 nm) and UVB (λ_{max} = 314.3 nm) stimuli (**Table 1, Figure 1F**). We found that while Cohort 1 was able to choose the UVA versus the UVB stimulus at a significant rate (89.5% success; Wilcoxon, V = 28, d.f. = 1, p = 0.022), Cohorts 2 and 3 were unable to differentiate between the UVB stimulus versus the UVA stimulus (65.1% success; Wilcoxon, V = 25, d.f. = 1, p = 0.341), or versus a dark stimulus (41.3% success; Wilcoxon, V = 10, d.f. = 1, p = 0.291). Attempts to train Cohort 4 to choose a dark stimulus versus a UVB stimulus were also unsuccessful (58.8% success; Wilcoxon, V = 27, d.f. = 1, p = 0.234). Cohort 1 was then used in a control experiment where the stomatopods were presented with an identical pair of UVA stimuli. The cohort's preference for the two stimuli was identical (46.1% preference for the left target; Wilcoxon, V = 12, d.f. = 1, p = 0.799). Finally, Cohort 1 was tested to again choose UVA versus a UVA-351 (λ_{max} = 351.1) stimulus, only 27.2 nm apart in maximum emission. The cohort remained able to choose the UVA stimulus at a significant rate (64.1% success; Wilcoxon, V = 35, d.f. = 1, p = 0.015). See **Table S1** for choice and participation data for each individual in these experiments. Over all the tests, the relative brightness of the stimuli did not have any effect on the choice of stimulus (GLMM, χ^2 = 1.2552, d.f. = 2, p = 0.534), however there was a clear difference in how the animals responded to the spectral pairs (GLMM, χ^2 = 16.913, d.f. = 2, p < 0.001) (**Figure S3**). The full dataset used in the GLMM analysis can be found in Supplementary Data File 1, with an explanation of its contents in the Supplemental Materials document.

In the trained choice assays we observed a trend in participation related to the UVB stimuli. Participation was markedly reduced in experiments where we attempted to train the stomatopods to choose the UVB stimulus (UVB vs. UVA, 22.3% participation; UVB vs. dark, 45.3% participation) (**Table 1**). A similar effect was also noted when a UVB stimulus was present as an alternative target stimulus (UVA vs. UVB, 56.5% participation, dark vs. UVB, 46.2% participation). When there was no UVB stimulus presented in the

experiment, the animals displayed an elevated rate of participation (UVA vs. UVA control, 95.7% participation; UVA vs. UVA-351, 69.9% participation).

Males and females did not differ markedly in the percent correct choices in any of these experiments (**Figure 2A**). However, males invariably showed much lower participation than females when there was a UVB stimulus present (**Figure 2B)** (males, 26.3% participation, n=17; females, 57.3% participation, n=17; Wilcoxon, $W = 55.5$, d.f. = 1, $p < 0.001$).

Innate Burrow Preference Tests

In the innate burrow choice tests, *H. trispinosa* showed significant aversion both to UVA (λ_{max} = 379.1 nm) and UVB (λ_{max} = 317.4 nm) stimuli (**Figure 1H, Table 2**). When presented with a choice between UV emitting burrows and a dark burrow, they chose the dark burrow at a significant rate: 83.3% preference versus UVB (Binomial test, number of dark burrow choices = 35, number of choices = 42, $p < 0.001$), and 74.4% preference versus UVA (Binomial test, number of dark burrow choices = 32, number of choices = 43, $p < 0.001$). Based on the apparently greater aversion to UVB stimuli observed in the trained choice tests, we hypothesized that when given an intensity-matched choice between burrows emitting UVA versus UVB light, *H. trispinosa* would prefer UVA emitting burrows. However, we found no significant preference in this case (54.2% preference for UVB; Binomial test, number of UVB burrow choices = 13, number of choices = 24, $p = 0.838$). When both burrows were dark, *H. trispinosa* showed no significant side preference (55.0% preference for the left burrow; Binomial test, number of left burrow choices = 11, number of choices = 20, $p = 0.824$). We also observed depressed participation in the UVA versus UVB experiment (52.2%) compared to experiments with a dark burrow option (UVA vs. dark, 80.8%; UVB vs. dark, 75.4%; and dark vs. dark, 74.1%).

DISCUSSION

The behavioural experiments demonstrate spectral discrimination within the UV range in the stomatopod species *Haptosquilla trispinosa*, regardless of brightness cues (consistent with previous experiments at human visible wavelengths showing that this species does not appear to use brightness cues when making colour discriminations [14]). The results of the trained choice experiments demonstrated that *H. trispinosa* could learn to choose a UVA stimulus against a UVB stimulus, but not *vice versa* (**Figure 1F,**

Table 1). This result could be interpreted as *H. trispinosa's* simply being unable to detect UVB light. However, the experiments also revealed a depressed participation rate when we attempted to train the stomatopods to choose a UVB stimulus, or simply when a UVB stimulus was presented as an alternate target choice (**Figure 1F**). This observation led us to hypothesize that *H. trispinosa* could discriminate the UVB and UVA stimuli from one another, but innately treated the UVB stimulus as an aversive cue and could therefore not learn to associate it with a food reward. We confirmed the ability of *H. trispinosa* to detect UVB by the innate burrow choice experiments, where we found that individuals consistently chose a dark burrow over a UVB-emitting burrow (**Figure 1H, Table 2**). We further hypothesized that when given the choice of a UVA- versus UVB-emitting burrow, the stomatopods would prefer the UVA-emitting burrow. However, we instead found that they had no preference and diminished participation in this case, suggesting that they simply do not like any brightly lit burrows. Taken together, these experiments show that *H. trispinosa* can discriminate UVA and UVB cues. However, they appear to treat the cues differently in the two behavioural contexts; being averse to the UVB stimulus in predatory behaviours and avoiding both UVA and UVB in shelter seeking behaviours. Furthermore, the depressed participation by males in the trained choice tests when the UVB stimulus was present suggests at a sexually dimorphic response to UVB cues (**Figure 2**).

UV sensitivity and UV-cue-driven behaviours are common amongst animals (reviewed in [21]), but the majority of examples involve UVA photoreception. Recently, behaviourally relevant UVB sensitivity has begun to receive some attention [22-24]. For instance, thrips display a UVB-specific phototactic response for an unknown purpose [22], and jumping spiders use UVA and UVB reflective patches in conjunction as sexual signalling cues [23, 24]. Our results offer an aquatic example of UVB behavioural sensitivity, which is somewhat surprising since UVB light is rapidly attenuated in water [21]. However, many stomatopods, including *H. trispinosa*, live in shallow, clear tropical waters with abundant UVB irradiance.

It is not well understood how stomatopods process colour information, or whether UV photoreceptors are integrated into the longer-wavelength colour processing system. The subset of photoreceptors responsible for UV sensitivity (the R8s) project directly to the medulla, bypassing the lamina where the projections of the R1-7 receptors (maximally sensitive to wavelengths of light between 400 and 700 nm) terminate, and where spectral comparison is thought to be initiated [25]. Interestingly, we found that stomatopods could discriminate cues within the UVA range that emitted light maximally at 378.3 nm (UVA) and 351.1 nm (UVA-351), only 27.2 nm apart in maximum emission (**Figure 1F, Table 1)**. This spectral discrimination

performance is comparable to the capabilities of this species at human-visible wavelengths [14]. Since only the midband row 1 R8 absorbs strongly in this region, it is likely that this discrimination is facilitated in conjunction with the third R8 receptor (maximally sensitive around 325 nm, **Figure 1C**, dashed grey line) or the R1-7 receptors. One of these R1-7 receptors, the midband row 4 distal main rhabdom receptor, is maximally sensitive at 420 nm but overlaps significantly in sensitivity with the row 1 R8, down to around 370 nm [8]. This suggests the potential for spectral comparison between the R8s and main rhabdom receptors in the midband.

Recent wavelength discrimination experiments in *H. trispinosa* at human-visible wavelengths (400-700 nm) have implied that stomatopods may be using a novel form of chromatic processing, recognizing narrow bins of wavelengths in a manner that can be likened to a spectral barcode scanner [14]. Such a system could rapidly encode and assess specific colour patterns, such as the resplendent markings found on many species of mantis shrimp. It remains to be seen how the UV-sensitive photoreceptors would contribute to such a chromatic processing system. However, our results demonstrating UV discrimination and UVB-specific aversion raises the exciting potential for the presence of UVB-encoded aggression cues on stomatopods that could serve as a robust and covert means of identifying one another's intentions before coming to blows.

ETHICS STATEMENT

Research using *Haptosquilla trispinosa* was carried out under the supervision of the staff of Lizard Island Research Station and with the following requisite permits: Australian Marine Parks (GBRMPA) permit nos. G12/35005.1, G14/36625.1 and Fisheries Act no. 140763.

DATA ACCESSIBILITY STATEMENT

The datasets supporting this article have been uploaded as part of the supplementary material.

COMPETING INTERESTS

We have no competing interests.

AUTHOR CONTRIBUTIONS

MJB designed the project, carried out the experiments, analyzed the data, and edited the manuscript. NWR analyzed the data and edited the manuscript. TWC assisted in experiments, supervised the project, and edited the manuscript.

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TABLES

Table 1. *Haptosquilla trispinosa* trained choice results. The "exp. stimuli" column indicates the wavelength that each cohort was trained to choose listed first. UVA, λ_{max} = 378.3 nm; UVB, λ_{max} = 314.3 nm; UVA-351, λ_{max} = 351.1 nm. *N*, number of individuals in each cohort. Percent participation (% part.) and percent correct choices (% cor.) are calculated by averaging the percent participation and percent correct choices for each individual in each experiment (see **Table S1** for full choice results for all individuals). P-values are calculated using percent correct choices for each individual within each experiment in a Wilcoxon signed rank test with continuity correction ($P_0 = 0.5$, $\alpha = 0.05$).

exp. stimuli	cohort	*N*	% part.	% cor.	Wilcoxon p-value	
UVA vs. UVB	1	7	56.5	89.5	**0.0215**	*
UVB vs. UVA	2	9	22.3	65.1	0.3411	*ns*
UVB vs. dark	3	8	45.3	41.3	0.2912	*ns*
dark vs. UVB	4	10	46.2	58.8	0.2340	*ns*
UVA vs. UVA[a]	1	7	95.7	46.1 (L)	0.7988	*ns*
UVA vs. UVA-351	1	8	69.9	64.1	**0.0156**	*

[a] In control experiments the same cue is present at both targets. "L" or "R" denote if the animal chose the left or right option.

Table 2. *Haptosquilla trispinosa* burrow preference experimental results. UVA, λ_{max} = 379.1 nm; UVB, λ_{max} = 317.4 nm; P-values are calculated with a binomial test ($P_0 = 0.5$, $\alpha = 0.05$).

exp. stimuli	preferred	alternate	choices	DNP [b]	trials	% part.	% pref.	p-value	
UVB vs. dark	35 (dark)	7 (UVB)	42	10	48	80.8	83.3	**1.51×10^{-5}**	*
UVA vs. dark	32 (dark)	11 (UVA)	43	14	54	75.4	74.4	**1.91×10^{-3}**	*
UVB vs. UVA	13 (UVB)	11 (UVA)	24	22	46	52.2	54.2	0.838	*ns*
dark vs. dark [a]	11(L)	9 (R)	20	7	35	74.1	55.0	0.824	*ns*

[a] In control experiments the same dark cue is present at both burrow options. "L" or "R" denote if the animal chose the left or right option.

[b] Did not participate: Did not directly enter one of the choice burrows within the experimental period.

381 **FIGURES LEGENDS**

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383 **Figure 1. *Haptosquilla trispinosa* UV behavioural assays.**

384 **(A)** *H. trispinosa* pictured in a natural burrow. Photo: Roy Caldwell. **(B)** An *H. trispinosa* eye with the dorsal
385 hemisphere (DH), ventral hemisphere (VH), and midband (MB) labelled. **(C)** Spectral sensitivities of the three
386 *H. trispinosa* UV sensitive photoreceptors, adapted from electrophysiological recordings in Thoen et al. [14].
387 UVA and UVB regions of the spectrum are indicated. **(D)** A diagrammatic cross section through the midband
388 of the eye. Ommatidial components: R1-7, retinular cells 1-7; R8s, retinular cell 8; CC, crystalline cone; Co,
389 cornea. R8 cell colour corresponds to spectral sensitivities in C, and their locations are inferred from typical
390 opsin expression patterns [10, 26] and UV filter pigment localization [11]. **(E-H)** Results of trained predatory
391 choice (E-F) and innate burrow preference (G-H) behavioural assays. E and G show simplified diagrams of
392 the respective experimental setups with normalized radiance spectra of their stimuli displayed to the left. Full
393 schematics and additional details can be found in **Figure S1**. Shaded colour regions correspond to R8 spectral
394 sensitivities in C. The bar graphs display average correct choice percent in the trained choice tests (**F**, black
395 bars) and burrow preference percent (**H**, black bars), as well as respective percent participation (thin, white
396 bars). Both bar graphs are labelled with circles indicating their respective stimuli corresponding to the spectral
397 plots in E and H. Black circles indicate a dark stimulus. Trained choice significance in E is calculated using a
398 Wilcoxon signed rank test with continuity correction from the percent correct choices for each individual in
399 the cohort ($P_0 = 0.5$, $\alpha = 0.05$; Table 1). Innate burrow preference significance in H is calculated with a
400 binomial test ($P_0 = 0.5$, $\alpha = 0.05$; Table 2).

401

402 **Figure 2. Male (blue bars) and female (red bars) correct choices (A) and participation (B) in trained**
403 **choice experiments from Figure 1F.** Symbol circles for tests are as in Figure 1F. Percentages are derived
404 from pooled choice data for each gender (Table S1).

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